Judges' choice

I ALDOSTERONE MEDIATES ANGIOTENSIN II INDUCED INTERSTITIAL CARDIAC FIBROSIS VIA A Nox2 CONTAINING NADPH OXIDASE

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Background: NADPH oxidases are major sources of reactive oxygen species. We previously showed that a Nox2 NADPH oxidase is critical for the development of angiotensin II (AngII) induced cardiac hypertrophy and fibrosis. In this study, we investigated the potential role of aldosterone (ALDO) in this process.

Methods: Mice lacking Nox2 (KO) and matched wild type controls (WT) were infused with either vehicle or AngII (1.1 mg/kg/day) for 2 weeks. Some animals received spironolactone (SPIRO, 200 mg/kg/day) in chow. A further group of mice underwent unilateral nephrectomy and were infused ALDO (0.2 mg/kg/day) or vehicle for 2–4 weeks together with 1% NaCl, 0.3% KCl in drinking water (ALDO/salt), n≥6 all groups.

Results: Interstitial cardiac fibrosis increased significantly with Ang II in WT (7.2 (SD 0.7) % to 11.5 (SD 1.0) %; $p<0.05$) but not in KO (6.0 (SD 0.6) % to 5.8 (SD 1.0) %, p = NS). The AngII-induced increase in fibrosis was inhibited by SPIRO (7.5 (SD 1.0) % *v* 6.6 (SD 1.0) % in control; p = NS). Expression of fibronectin and procollagen I mRNA increased in AngII treated WT by 2.9 (SD 0.6) and 3.0 (SD 0.7) fold respectively (both $p<0.05$). SPIRO partially inhibited the increase in procollagen I (1.9 (SD 0.2) fold, $p<0.05$) but not fibronectin (2.8 (SD 0.3) fold, p = NS) in WT mice. In line with these results SPIRO inhibited the increase in NADPH oxidase activity produced by AngII in WT from 5.1 (SD 0.4) to 3.3 (SD 0.1) integrated light units (ILU), $p<0.05$. Fibrosis increased in the WT ALDO/salt group after 4 weeks (12.0 (SD 1.7) % *v* 6.3 (SD 0.3) %; $p<0.05$) but not in KO (5.8 (SD 1.0) % *v* 6.8 (SD 0.8) %, p = NS). This was associated with an increase in procollagen I and fibronectin mRNA in WT (2.1 (SD 0.3) and 1.9 (SD 0.2) fold respectively, both $p<0.05$) but not KO mice (1.1 (SD 0.1) and 0.9 (SD 0.1) fold respectively, both p = NS). Myocardial NAPDH oxidase activity was increased in ALDO/salt WT (4.2 (SD 0.3) *v* 3.3 (SD 0.2) ILU, $p<0.05$) but not KO (4.6 (SD 0.2) *v* 4.5 (SD 0.3) ILU, p = NS).

Conclusion: These data suggest that the profibrotic effect of AngII in the heart is mediated through aldosterone and involves activation of a Nox2 containing NADPH oxidase.

II RANDOMISED CONTROLLED TRIAL COMPARING THE CLINICAL EFFECTIVENESS OF HOME BASED AND HOSPITAL BASED REHABILITATION AFTER ACUTE MYOCARDIAL INFARCTION: THE CORNWALL HEART ATTACK REHABILITATION MANAGEMENT STUDY

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Background: Comprehensive cardiac rehabilitation (CR) after acute myocardial infarction (AMI) is considered to be an effective intervention. Participation in CR remains sub-optimal and practical problems involved in accessing hospital based rehabilitation (hosp-CR) may be a contributory factor. Home based rehabilitation (home-CR) programmes are available in the UK but the relative effectiveness of home-CR has not been established.

Objectives: To measure the clinical effectiveness of a home-CR programme compared with hosp-CR in patients following AMI. Design: randomised controlled trial. Interventions: six week nurse facilitated self-help package of home-CR (the Heart Manual) or attendance at outpatient hosp-CR classes for 6–8 weeks.

Main outcome measures: Hospital Anxiety Depression scale (HADS), Quality of Life after Myocardial Infarction (QLMI) questionnaire, total serum cholesterol (TC), and exercise capacity on treadmill testing (TT).

Methods: 104 patients (age 63 (SD 11) years, 84 males) with uncomplicated AMI and without major comorbidity were randomised to either home-CR (n = 60) or hosp-CR (n = 44). All outcomes except TT were measured at baseline, 3, and 9 months.

Results: Using an intention to treat analysis, the primary outcome measure at 9 months (HADS depression score) showed no significant difference between home-CR and hosp-CR (ANCOVA adjusted mean difference 0.75, 95% CI −0.47 to 1.97; p = 0.225). Statistically significant improvements in mean scores between baseline and 9 months were observed in both groups: global QLMI (home-CR 1.01, 95% CI 0.62 to 1.41; $p<0.0001$; hosp-CR 0.94, 95% CI 0.58 to 1.30; $p<0.0001$), and in TC (home-CR −1.35 mmol/l, 95% CI −1.69 to −1.00; $p<0.0001$; hosp-CR −1.15 mmol/l, 95% CI −1.55 to −0.74; $p<0.0001$). The home-CR group showed significant improvement in TT (METs, metabolic equivalents) from 3 to 9 months (0.87, 95% CI 0.35 to 1.39; p = 0.002) compared with hosp-CR (0.46, 95% CI −0.31 to 1.23; p = 0.23). However, the improvements in HADS, QLMI, TC, and TT did not differ significantly between the two groups.

Conclusion: Home-CR using the Heart Manual is as effective as hosp-CR for patients after AMI. Significant improvements in quality of life and prognostic risk factors occurred after both CR interventions. This study lends support to the policy of offering home-CR as an alternative to hosp-CR. This will increase patient choice and is likely to improve the current low uptake of CR.

III CARDIAC nNOS REGULATES MYOCARDIAL RELAXATION BY STIMULATING PHOSPHOLAMBAN PHOSPHORYLATION

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Introduction: We have shown that selective gene deletion of the neuronal nitric oxide synthase (nNOS) results in a significant impairment in basal and adrenergic myocardial relaxation. However, the mechanism underlying these findings remains unclear.

Methods and Results: To address this question, we studied relaxation and [Ca]i reuptake in LV myocytes from nNOS knockout (KO) mice and wild type littermates (WT). The decay of the field stimulated [Ca]i transient was prolonged in nNOS KO myocytes both at baseline and in the presence of ISO (1 μmol/l), but the decay of the caffeine induced [Ca]i transient (which does not depend on SR Ca reuptake) did not differ between groups. Similarly, disabling SR function with thapsigargin abolished differences in relaxation between KO and WT mice. These data suggest that slower SR Ca reuptake may be responsible for the impaired myocardial relaxation in nNOS KO mice. Phosphorylation by either protein kinase A (on Ser16) or calmodulin kinase II (on Thr17) removes the inhibitory action of phospholamban (PLB) upon the SR Ca pump (SERCA) and accelerates relaxation. Quantitative immunoblotting of LV homogenates or LV myocyte membrane subfractions showed a significant reduction in PLB phosphorylation at the Ser16 and Thr17 sites in KO mice versus WT (n = 5 hearts per group, $p<0.01$). Total PLB was modestly decreased in nNOS KO (by 28%) but the PLB Ser16/PLB ratio remained significantly lower in these mice (ca. 40% of WT). SERCA protein level did not differ between groups. nNOS specific inhibition with SMTC (100 nmol/l) caused a significant reduction in Ser16 and Thr17 PLB phosphorylation in WT mice, with no change in total PLB. Pretreatment of isolated LV myocytes with ISO (1 μmol/l) increased Ser16 PLB phosphorylation in both groups, but the PLB phosphorylated fraction remained significantly lower in nNOS KO myocytes. Co-immunoprecipitation indicated a physical interaction between nNOS and Ser16 PLB but not with SERCA.

Conclusion: These findings indicate that nNOS is an important dynamic regulator of PLB phosphorylation and as such of SERCA activity and suggest that upregulation of myocardial nNOS in infarcted hearts may be an important adaptive response aimed at preserving SR Ca cycling.

IV RAPID, SAFE, AND EFFECTIVE MANAGEMENT OF ACUTE CHEST PAIN IN A DEDICATED CHEST PAIN ASSESSMENT UNIT AT ST JAMES'S HOSPITAL, DUBLIN

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Background: Chest pain is the second most common complaint seen in the emergency department. International figures show that up to 8% of these patients are inappropriately discharged. Inappropriate discharge is associated with fivefold increase in mortality (26%). To address this problem we established a four bedded fully monitored chest pain assessment unit located immediately adjacent to the emergency department. The model used includes continuous ST-segment and arrhythmia monitoring, rapid turnaround cardiac biomarkers, and exercise stress testing before discharge after 12 hours. Finally the patients returned for a further clinical review after 48 hours.

Methods and Results: Over a two year period 1629 patients were admitted to the CPAU. 57% were male and 43% female. The mean age was 49 and 54 years respectively. 73% of patients were safely discharged within 12 hours of assessment. 15% of patients had an

abnormal assessment and were transferred to the cardiology service. 12% of patients had other medical conditions such as pulmonary emboli accounting for their chest pain and were managed appropriately. Of those who were transferred to the cardiology service 64% of these underwent early (within 48 hours) diagnostic angiography. 60% of these had abnormal results. 47% of males and 35% of females with abnormal angiographic studies went on for PTCA. 42% of males and 64% of females were managed medically. 12% of males and 2% of females went on for coronary artery bypass surgery respectively. Of those patients discharged with a negative assessment 100% had a negative 48 hour Troponin T test. There were no adverse events from early EST, although all patients had a negative 12 hour Troponin before their EST.

Conclusion: We have demonstrated that the implementation of a designated chest pain assessment unit, which is run collaboratively between the emergency medicine and cardiology service, allows for enhanced acute care for patients presenting with chest pain of possible cardiac origin, together with safe early discharge.

V PREVENTION OF HYPOXIA-INDUCED PULMONARY HYPERTENSION AND VASCULAR REMODELLING BY TARGETING ENDOTHELIAL TETRAHYDROBIOPTERIN-DEPENDENT NITRIC OXIDE SYNTHASE REGULATION

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Introduction: Pulmonary hypertension (PH) is a fatal disease, characterised by reduced nitric oxide bioactivity, vasoconstriction, and vascular remodelling. We investigated the potential importance of tetrahydrobiopterin (BH4), a critical cofactor that regulates nitric oxide synthase (NOS) activity, in the pathogenesis of hypoxia induced PH.

Methods: We evaluated the responses to both acute and chronic hypoxia in mice with targeted endothelial overexpression of GTP-cyclohydrolase I (GCH), the rate limiting enzyme in BH4 synthesis.

Results: Lung BH4 levels were doubled in transgenic (GCH-Tg) mice compared with WT littermates (7.04 (SD 0.66) *v* WT 3.83 (SD 0.46) pmoles/mg protein, $p<0.01$), and were unchanged following chronic hypoxia (10% O_2 for one week). Lung eNOS protein levels, quantified by immunoblotting, were similar in both genotypes. However, NOS activity, determined by conversion of 14C-arginine to 14C-citrulline in lung homogenates, was doubled in GCH-Tg mice ($p<0.001$). Vasomotor responses to acute hypoxia were quantified in perfused lung preparations. Pulmonary vasoconstriction to acute hypoxia (2% O_2 for 10 minutes) was significantly reduced in GCH-Tg mice (2.6 (SD 0.4) *v* WT 4.3 (SD 0.2) mm Hg, $p<0.05$). Right ventricular systolic pressure (RVSP), assessed by direct cardiac puncture, was normal in both genotypes during normoxia, but the rise in RVSP following chronic hypoxia in WT was prevented in GCH-Tg mice (24.8 (SD 1.9) *v* WT 29.6 (SD 1.2) mm Hg, $p<0.001$). Accordingly, the right ventricular (RV) hypertrophy seen in WT mice exposed to hypoxia was completely normalised in GCH-Tg mice (RV/LV ratios: 0.26 (SD 0.01) *v* WT 0.33 (SD 0.01), $p<0.001$). Vascular remodelling, assessed by % of thick walled peripheral vessels immunostained for smooth muscle actin, were similar in GCH-Tg and WT in normoxia, but the increase in WT mice after hypoxia was abolished in GCH-Tg mice (23.6 (SD 2.4) % *v* WT 49.5 (SD 1.5) %, $p<0.001$).

Conclusion: Endothelial BH4 augmentation enhances NOS activity, limits the immediate constrictor response to acute hypoxia and prevents chronic hypoxia induced PH, RV hypertrophy, and vascular remodelling. BH4 dependent endothelial NOS regulation is a potential therapeutic target in pulmonary hypertension.

VI COMPARISON OF FIRST AND SECOND ACUTE MYOCARDIAL INFARCTION: RECENT TRENDS IN INCIDENCE AND CASE FATALITY

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Introduction: Recent studies have shown that the incidence of first acute myocardial infarction (MI) is falling and survival after first events is improving. Nothing, however, is known about contemporary trends in either the incidence of or prognosis after a second MI. The aim of this study was to describe case fatality following a second MI, compared with a first MI, and to evaluate whether, after a second event, patients have experienced similar improvements in survival to those suffering a first MI.

Methods: Using the Scottish Linked Morbidity Record Database we identified all patients with a first or second MI between1990 and 2000. We compared baseline characteristics and analysed case fatality up to 5 years. We used multivariate modelling at 30 days and 5 years to examine factors affecting prognosis in men and women and to determine trends over time.

Results: There were 110 226 admissions with a first MI and 9664 with a second MI. The median interval between a first and second MI was 2 years. While the number of admissions with a first MI decreased by 28%, the number with a second fell by 58%. Overall crude case fatality following a first MI (second MI) was 20.1 (24.5) %, 28.4 (38.3) % and 44.7 (60.2) % at 30 days, one year and five years respectively. Overall median survival following a first MI (second MI) was 8.8 (3.6) years in men and 4.3 (1.8) years in women. After a first (second) MI, adjusted 30 day case fatality fell by 38 (21) % in men and 24 (17) % in women. After a first (second) MI, adjusted longer term case fatality fell by 27 (29) % in men and 23 (17) % in women.

Conclusion: The striking decline in second infarctions is likely to reflect improvements in secondary prevention. Short term case fatality has improved less after a second MI than after a first MI but there has been a comparable improvement in longer term survival. Despite this prognosis remains much worse after a second than first MI and every effort should be made to prevent recurrent infarction.